Production of Rapid Growth and Specific Pathogen Resistant (SPR) Broodstock of *Litopenaeus vannamei* and Application of β-Glucan on Shrimp Aquaculture

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ABSTRACT

The present study attempted to establish a system for the production of rapid growth and specific pathogen resistant (SPR) broodstocks of Pacific white shrimp (*Litopenaeus vannamei*). The studies were focalizing on the production of white spot disease virus (WSSD) resistant shrimps. The results obtained from the present study showed that shrimps derived from WSSD resistant broodstocks revealed a significantly higher survival rates than those of the control shrimps. It was also showed that shrimp obtained from rapid growth strain of *L vannamei* showed a better growth rates than those of the regular shrimp. The study on the investigation of efficacy of β-Glucan produced from various fungi including *Schizophyllum commune* and *Ganoderma lucidum* and *Agaricus blazei* showed a significant contribution on the survival of cultured *L. vannamei*.

Key words: Repid growth, β-Glucan, Survival, Growth, *Litopenaeus vannamei*.

INTRODUCTION

Following culture techniques of Pacific white shrimp (*Litopenaeus vannamei*) has been successfully established in Taiwan in the year of 1998, this species has become one of the most important cultured shrimps in Asia. In the year of 2001, the production for this species upgraded to 100 thousand and 10 thousand metric tonnes in China and Taiwan respectively. It is anticipated that the production for *Litopenaeus vannamei* will significantly increase up to 2,000-3,000 thousand and 200 thousand metric tons in these two areas in the year of 2005. For sustainability of Pacific white shrimp culture, developing techniques for a stable production and preventing the occurrence of diseases for this species are considered to be the most important. Amongst various factors that affect the stable production of Pacific white shrimp, elimination of viral infectious diseases and uses of fast growth seed were recommended as key factor to affect the success of *L. vannamei* culture. There is an urgent need in developing systems for culture of specific pathogen resistant (SPR) and fast growth strain of *L. vannamei*.

The present study attempts to develop a system for growing or selecting of rapid growth and specific pathogen resistant (SPR) Pacific white shrimp (*Litopenaeus vannamei*).

It is hoped that through the accomplishment of the study, the technologies for SPR shrimp may effectively developed in Taiwan so that sustainability of the shrimp aquaculture will become possible. The present study also aims to investigate the potentiality for the SPR and fast growth shrimps.

Growing shrimps are usually under the stresses from environment, food, the excessive culture density of animal itself and the challenge of the pathogens. Diseases of cultured shrimps may cause mass economic losses. To obtain a better survival and harvesting for shrimp, uses of drugs and vaccines is the only strategy in the past. However, in recent several years, drug or vaccines become less effective and over uses of synthetic chemicals has already threaten to the human health. In a meeting entitled "Monitoring anti-microbial usage in food animals for the protection of human health" held in 2001 in Oslo, Norway by World Health Organization (WHO) strongly suggested that uses of drug for food animals should be stickly regulated. Due to public health problem arisen from contaminated toxic chemicals in food animals, many countries have used drug residue existing in food animals as a trade barrier. For this reason, shrimp culture industry is considered to be a very high risk investment. It is therefore urgently needed to look for a non-toxic and total safe strategies for the industry.

Following long term investigation, it is concluded that uses of natural immunostimulant is one of the best strategies to ensure a sufficient survival rate as well as a better quality of product. Amongst a very few immunostimulant used it is demonstrated that β -Glucan especially β 1,3, β 1,6-D-Glucan may effectively enhance the natural defenses of domestic and aquatic animals against all kind of negative challenges. The present study therefore attempts to investigate the efficacy of β -Glucan on immune-enhancing activities through evaluation of hemocyte variation in hemolymph and variation of superoxide disunities (SOD) in hepatopancreas was also evaluated. The survival of culture shrimps (*Litopenaeus vannamei*) following β -Glucan ingestion was also evaluated.

MATERIALS AND METHODS

The original seeds of experimental shrimps (*Litopenaeus vannamei*) used in the present were obtained from Hawaii USA. For the evaluation of specific pathogen resistace (SPR) and rapid growth, 2nd generation obtained from the pond infected with WSSD were selected. After mass motality due to WSSD, the existing shrimps were continuously cultured for 6 months then the groups of biggest shrimps were collected for the hatchery and experimental uses. All the larvae obtained from the above described strains were cultured in a 30-liter aquaria than the growth rates and the challenge infection by feeding of white spot diseased shrimp was performed. The growth rate was calculated by weighting the body weight at the 4, 8, 12, 13, 16 and 20 weeks after cultivation. The capability of white spot disease resistant of *L. vannamei* was performed by calculating survival rates at three week after feeding of white spot disease shrimp muscles. The shrimps obtained from regular hatchery using the broostocks obtained from local cultured ponds were used as control experiment. A recepis of artificial shrimp feed as desoribed in Table 2 was designed and tested for the efficacy on the enhance of maturation of shrimp.

To evaluate the efficacy of β -Glucan variation of hemocyte and superoxide dismutase (SOD) activity following ingestion of β -Glucan were performed. β -Glucan derived from mushrooms including *Schizophyllum commune*, *Ganoderme lucidum* and *Agarics blaze* at a concentration level of 70% was supply by Perfect Health Biotechnology Co. Ltd, in Taiwan. For comparison, sodium alginate and laminarin (extracted from *Laminaria digitata*) obtained from Sigma, U.S.A were used.

The experiments were divided into 21 groups each group contains 40 shrimps. Each group feeded with feed containing 0, 0.1% or 0.4% of β -Glucan, sodium aginate and laminarin respectively. 0, 1, 2, 4, 8, 16, 30 days following ingestion of β -Glucan, sodium aginate and laminarin, 5 shrimps were sacrificed for each group at each time interval. The samples of hemolymph was collected and hemocyte in hemolymph was also counted. For SOD examination, hempatopancrease from sacrificed shrimps were also collected.

The collected hepatopacrease was processed for superoxide dismatase, SOD assay using RANSOD Kit (Randox).

To evaluat the efficacy of β-Glucan in the field, β -Glucan (70% concentration) supplied by Perfect Health Biotechnology, Co. Ltd in Taiwan was used as feed additive at concentration level of 0.5%. Three ponds containing approximately 3-5$\times 10^4$ shrimps were feeded with the obove experimental feed. No β-Glucan feed was used as control experiments. Following 5 month cultured periods, survival and growth rates are calculated for both experimental and control ponds.

RESULTS

Results presented in Table 1 and Fig.1 showed that the larval shrimps of *L. vannamei* obtained from SPR broodstocks showed a significantly higher survival rates than those of the control shrimps. It is suggested the broodstock collected from SPR selected broodstock may show a very significant resistance against white spot disease virus. It is also showed that the larvae derived from broodstock of bigger size may reveal a rapid growth rates than those of derived from the broodstock in regular size.

It may suggest that the selection of size and SPR broodstocks is impartant for *L. vannamei* aquaculture.

To achieve a complete aquaculture systems for Pacific white shrimp (*Litopenaeus vannamei*), artificial broodstock feed as deseribe in Table 2 was used for the availability of mature broodstock of *L. vannamei*. The results showed that using of the artificial broodstock feed containing the described ingredients, an enhancement of maturation for *Litopenaeus vannamei* can be achieved.

Table 1. Survival of white spot disease resistant shrimps and control after challenge of pathogen.

	Experiment No.	Survival No.	Survival Rates
Control Group A	1000	352	35%
Control Group B	1000	283	28%
SPR A	1000	724	72%
SPR B	1000	651	65%

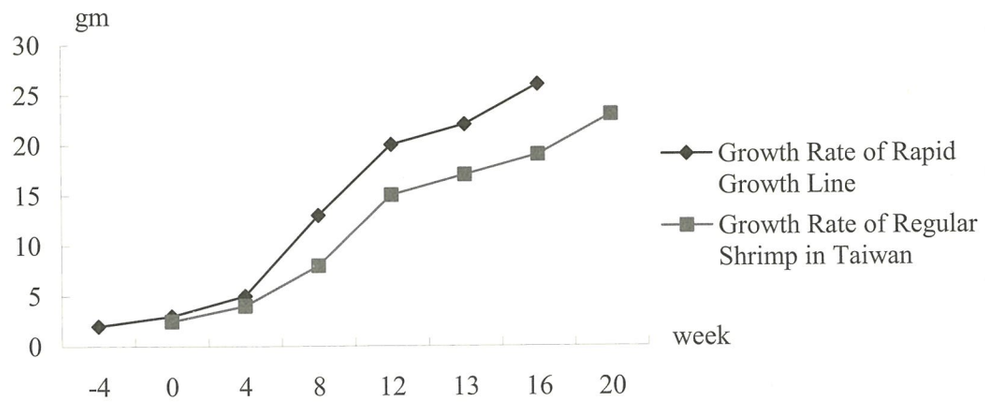

Fig.1. Growth Rates of Selected Rapid growth shrimps and the Control shrimps.

Table 2. Main Ingredients for Artificial Broodstock Feed of *Litopenaeus vannamei.*

Ingrechinet	%	Ingrechinet	%
shrimp meal	10	cholesterol	0.5
fish meal	48.4	astaxanthin	0.1
squid meal	10	vit. D3	0.01
yeast	1	vit. E	0.5
α -starch	18	taurine	0.5
fish oil	5	mineral	2
lecithin	1	vitamin mix	2
choline chloride	1		

Into the above ingredients steroids including 17 α -hydroxyprogesterone、Progesterone and 17 β -estradiol at a ratio of 1:6:2 of were added.

Results presented in Table 3 show that when experimental shrimps feeded with the immunostimulants inclueding β -Glucan from mushrooms, laminarin and sodium arginate, a significant upgrade of hemocytes in hemolyph was available comparing to the control shrimps. However, the best results was obtained when the shrimps was feeded with mushroom β -Glucan. The results also showed that during the course of experiment, β -Glucan treated shrimps showed the highest hemocyte counts. The presence of superoxide dimutase (SOD) may indicate the capability of shrimps in defence of existing stressors. The experiments performed on the detection of SOD in hepatopancrease through oral ingestion of β -Glucan lamiuarin and sodium aginate respectively were showed in Table 4. The results indicated that the feed supplemented with β -Glucan reveals the best SOD enhancing capability than those supplemented with laminarin and sodium aginate. Experiments performed on the field for cultured Pacific white shrimps (*L. vanmaemi*) feeded with mushroom β -Glucan is showed in Table 5. The results showed that cultured shrimp treated with β -Glucan revealing a very significant higher survival rates comparing with that of the control shrimp. However, due to high survival rates, cultured density maintained higher, the experimented shrimps showed a slightly slower growth than that of the control shrimps.

Table 3. Total haemocyte count in the haemolymph of Pacific white shrimp (*L. vannamei*) fed diets with different immunostimulants during the culture period.

Treatment	Dose	Days						
	(g/kg)	0D	1D	2D	4D	8D	16D	30F
Control		200.7±22.2	185.8±12.9^{c}	208.8±41.4^{e}	207.0±15.8^{d}	215.3±28.4^{d}	221.7±2.6^{d}	204.7±7.1^{e}
β-glucan	1		508.8±51.8^{a}	775.7±37.0^{a}	375.7±21.0^{b}	627.2±33.6^{a}	393.0±10.0^{a}	452.2±41.0^{b}
	4		517.8±49.0^{a}	550.8±54.1^{b}	469.0±26.9^{a}	431.8±77.0^{b}	376.7±13.1^{a}	502.5±23.4^{a}
Laminarin	1		509.0±64.7^{a}	549.3±64.2^{b}	309.8±33.6^{c}	240.0±50.6^{cd}	314.0±51.3^{b}	401.2±38.5^{c}
	4		373.7±21.1^{b}	303.5±8.2^{c}	226.2±27.5^{d}	383.0±20.9^{b}	282.7±7.7^{bc}	335.0±4.2^{d}
Sodium alginate	1		332.3±25.4^{b}	286.7±30.3^{cd}	308.0±10.6^{c}	300.5±14.1^{c}	363.3±27.8^{a}	327.8±8.5^{d}
	4		229.2±45.9^{c}	219.2±14.6^{e}	288.3±32.4^{c}	248.2±26.5^{cd}	263.0±32.5^{cd}	236.8±24.4^{e}

Values represent mean, and SD. a,b,c: mean in the same column with the different letter are significantly different (P<0.05).

Table 4. Superoxide dismutase (SOD) activity (U/mg protein) in the hepatopancrease of the white shrimp (*L. vannamei*) fed diets with different immunostimulants duing the culture period.

Treatment	Dose	Days						
	(g/kg)	0D	1D	2D	4D	8D	16D	30F
Control		0.51±0.12	0.56±0.14^{a}	0.43±0.03^{c}	0.51±0.05^{d}	0.45±0.14^{cd}	0.51±0.08^{a}	0.60±0.09^{bc}
β-glucan	1		0.62±0.13^{a}	0.91±0.13^{a}	1.65±0.29^{a}	0.76±0.19^{b}	0.54±0.13^{a}	0.27±0.04^{e}
	4		0.64±0.12^{a}	0.75±0.13^{ab}	0.89±0.11^{b}	0.48±0.03^{cd}	0.52±0.09^{a}	0.81±0.09^{a}
Laminarin	1		0.56±0.06^{a}	0.46±0.04^{c}	0.83±0.09^{bc}	0.58±0.08^{bc}	0.54±0.08^{a}	0.67±0.05^{ab}
	4		0.55±0.10^{a}	0.59±0.18^{bc}	0.82±0.11^{bc}	0.61±0.10^{bc}	0.28±0.06^{b}	0.58±0.16^{bc}
Sodium alginate	1		0.52±0.11^{a}	0.57±0.09^{bc}	0.50±0.11^{d}	1.10±0.19^{a}	0.55±0.19^{a}	0.34±0.6^{de}
	4		0.51±0.22^{a}	0.88±0.11^{a}	0.65±0.05^{cd}	0.30±0.04^{d}	0.56±0.12^{a}	0.48±0.08^{cd}

Values represent mean, and SD. a,b,c: mean in the same column with the different letter are significantly different (P<0.05).

Table 5. Surved and Growth of Pacific white shrimp (*Litopenaeus vannamei*).

		Initial culture density	Survival (%)	Harvesting Size
Experiment	1	35000/pond	30,000(85.71%)	12.05**±2.5**
Experiment	2	42000/pond	35,000(83.33%)	12.12**±3.2**
Experiment	3	38000/pond	29,000(76.32%)	12.53**±2.1**
Control	A	42000/pond	15,000(35.71%)	13.45**±1.7**
Control	B	45000/pond	13,000(28.89%)	13.82**±3.2**

Artifical feed containing 0.5% of β -Glucan were used for 1-3 Experiments.

DISCUSSION

White spot disease virus (WSSV) is demonstrated to be virulent to penaeid shrimps such as *Litopenaeus vannamei* and *Penaeus monodon* and considered to be responsible for serious economic losses on shrimp farms throughout the world. (Wongteerasupaya *at al.*, 1995; Lightner, 1998 and Lightner *et al.*, 2001) Many strategies for the prevention of disease occurrence were therefore performed (Acre *et al.*, 2000; Argue and Alcivar-Warren, 2000 and Bennie, 1998). Uses of specific pathogen resistant bloodstock or larvae was considered to be one of the feasible ways to prevent the occurrence of mass mortality due to pathogenic infections (Tang *et al.*, 2003). Studies performed by Argue *et al.*, (2002), Gjedrem (1997) and Gjedrem and Finland (1995) indicated that selective breeding of pacific white shrimp (*Litopenaeus vannamei*) either growth rate or resistance to virus infection may contribute to the successful shrimp aquaculture. Similarily, the studies performed in the present study showed that larvae derived from naturally selected white spot disease virus (WSDV) resistant broodstock exhibited significant resistance against WSDV. It was also showed that significant higher growth rate was obtained for the larvae of rapid growth shrimps. It is concluded that significant improvements on shrimp aquaculture can be made in Pacific white shrimp (*Litopenaeus vannamei*) through selective breeding in rapid growth and specific pathogen resistance. The results obtained from the present study also showed the uses of artificial feed with various steroid hormones, the maturation for Pacific White Shrimp may be significantly enhanced. This study may suggest that a complete cultivation for rapid growth and disease resistant *L. vannamei* can be accomplished for *L. vannamei*.

It is generally recognized that using of drug has been considered to be one of the most important negative impact factors for shrimp aquaculture. To resolve disease problem, shrimp aquaculture has therefore been foculized on strengthening defence mechanism by using immunostimulants. Amongst various immunostimulants, lipopolysaccharide and extract from *Fusarium* has demonstrated to be effective in the upgrade of total hemocyte count (THC) (Johansson *et al.*, 2000) for shrimps. Results obtained from Cheng *et al.* (2003) has demonstrated that feeding of β-1,3-Glucan may significantly enhance THC in viral infected shrimps. Similarily, the present study also showed that feeding of sodium alginate, laminarin and mushroom β -Glucan may significantly upgrading THC in the experimental shrimp. Amongst the above described materials, β -Glucan showed a best result on the enhancement of THC in experimental shrimps. Superoxid dismutase (SOD) is an active enzyme which may effectively eradicate the free-radicals in shrimp. The results obtained in the present study also showed that using of β-Glucan in feed may significantly and rapidly upgrading SOD activity in shrimp. The result may suggest that β-Glucan possesses a superior immunomodulating capability for shrimps. High survival rate obtained from field showed that using of β-Glucan in feed may significantly equip the shrimp with defence tool for shrimps. It is reasonable to anticipate that using of β-Glucan should be one of the most useful strategies for the prevention of viral or bacteria epizootics for cultured shrimps.

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